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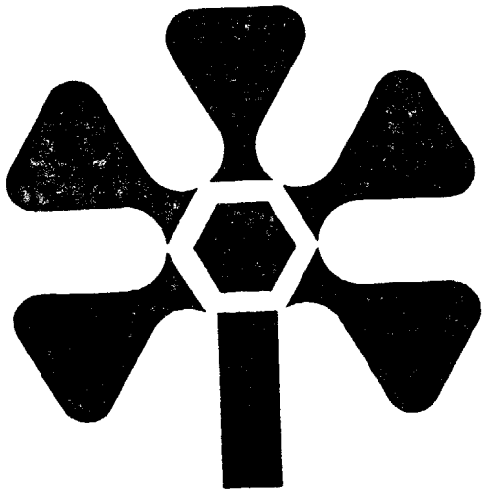

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FERMENTATION — A QUESTION OF LIFE

First of all, may I take just a moment to express my pleasure at visiting the University of Western Ontario on its lovely campus here. When I taught at the University of Wisconsin, whenever I did have occasion to drive east, I would always choose the scenic route via the Lake Michigan Ferry, Detroit and London, and visit some of my old friends here: I hope Dr. Robinow is in the audience today.

I am not going to be making any startling announcements of further breakthroughs; you will have heard a number of those during the course of these particular sessions. Instead, my remarks are rather what you would expect to hear at the closing end of the session, when you are a little too tired to absorb still one more concrete datum, and welcome a chance to relax and wind down. Unfortunately, the airplane schedules didn't cooperate with that, and so I am a little bit out of place at this front end of the program.

The program committee challenged me to aggregate some of my own thoughts on the strategies of further development in recombinant DNA technology. A session is being held right now in Toronto that is dedicated to futures; and I want to be sure that I am in the right symposium in the orientation of my remarks. My focus will be some rather manifest elements in the logic of the existing science and technology. This is moving so rapidly that we are contemplating a time horizon with a median of perhaps five, or at most ten years, for further developments. There will be some vexing exceptions; it isn't always possible to anticipate in advance just how hard a given problem is going to be: but a number of things can be foreseen with some confidence, as being the agenda of the next symposium of this kind within the next few years. There will be exceptions -- twenty years ago, many of us believed we would soon advance from the cloning of a frog from somatic cell into the egg, to the cloning of a mammal. That has so far proved not to be feasible.① Perhaps fundamental biological distinctions between frogs and mammals, of which we are still not fully aware, impede those kinds of developments. Similar pitfalls may affect the extrapolation of DNA technology. However, if we look at the recent past, if anything, the pace of events in the last five years has exceeded most people's prognostications, even my own. One loses one's breath trying to keep up with the pace, not only in scientific, but in technological developments in this field.

May I take a few things as given for this discussion, without depreciating the enormous ingenuity and diligence, insight and imagination that are involved in the development of the techniques? They are also very great fun, and I don't want to deprive anybody of the opportunity to engage in those details, even though I am going to leapfrog over all of them just now. My stipulation is that our appreciation of the structure of DNA and of a few of the enzymes, (I think we have far from exhausted them), that are involved in its replication, in recombination, in repair, in splicing and so forth, and in the regulatory factors that control those, permits us to plan the manipulation of DNA as if it presents only difficult but superable technical problems. It is very difficult for me to visualize an objective in DNA designs for which we cannot outline a plausible research program.

Our strategic challenge almost unique in the history of technology is less how than what!

Many techniques other than DNA-splicing and implantation into an appropriate vector are crucial for useful applications. They include the systematic fractionation of DNA, particularly from eucaryotes with large genomes, the isolation of particular components, with the best ways of expanding them; modifying those sequences in ways that open up new lines of experimentation with respect to the end products that one is going to see; the confrontation of products in a variety of metabolic pathways with one another, techniques for sequencing both in an analytical and in a synthetic sense the desired intermediate and end products. However, I repeat, those are today fairly manifest technical problems for which answers are being found every day. We can put it down that we have really a very thorough repertoire of procedures, either in hand or very shortly in hand to do whatever is socially and technically and economically feasible and desirable in those spheres.

As we work our way through these problems, and for example, find ways in which we can assure fairly consistent expression of a sequence, even at very low levels; we can of course then use the most sensitive, or selective procedures to isolate the rare progeny we seek. We are already much further along today than we were two or three years ago in this methodological infrastructure.

How then we manage the development of this technology? Our orientation should not be confined to products themselves, but also how these can be introduced, marketed, integrated into our science and technology and our economy. These issues deserve at least a small percentage of the time that is now occupied with the technological feasibility of specific ventures. Please recall too that DNA splicing is just one of a host of techniques for doing genetic experimentation, which began in a systematic way about 115 years ago with Mendel. Hybridization is still the bedrock of genetic analysis in all organisms: recombining DNA in the way that nature had evolved will still play a very large part in all that we do in this field in a very wide variety of organisms. Still other technologies have to be thought about: the development of chimeric or mosaic embryos, organisms which contain not admixtures of DNA within a single nucleus but the allophenic mice that Beatrice Mintz is making, where the embryos are constituted from zygotes of a variety of origins. This opens up another modality of "genetic engineering", if you like, it beins to breach the boundary between the somatic and the germ line in a given animal, permits the introduction of genetic innovations into a germ line that would not otherwise be feasible. This can go on over a wide variety of other ontogenetic technologies.

The one development that I would neither advocate nor foresee in any practical way is the direct application of gene modification to human beings. There has been an enormous amount of nonsense about that, both at the level of technical feasibility and also of moral, political and practical and common sense feasibility. One has to stop and think what one would have to do to validate a procedure that one would wish to advocate for the rectification of genetic disease in an already existing individual suffering from a genetic defect. I think one sees immediately the impossibility of the situation. How would you ever set up a clinical trial that would enable you to demonstrate that injecting DNA or any other such material into, say, a child with sickle cell disease or cystic fibrosis or phenylketonuria was an efficacious and safe procedure, particularly in the face of competing

techniques that have a higher degree of predictability and assurance and reliability - and I refer here particularly to pre-natal diagnosis and preemptive abortion? I think the moral imperative to prevent the birth of defective children by our insight into the inevitability of genetic disease in certain confrontations, in our ability to monitor those pregnancies with more and more specific and reliable methods for knowing just what the outcome of a particular gestation is going to be, is an ethical categorical that overrides the possibility of the clinical trials for the rectification of disease, once established.

On the other hand we are going to learn a great deal about diagnostic human genetics, the understanding of the way in which gene alterations result developmentally and physiologically into a line of defect, and how these can be mitigated by therapeutic measures as well as by the preemptive ones that I have just indicated. Diagnosis will be the main role DNA of DNA technology applied to humans.

Even more than the products we surely will make, the applications to industrial microbiology, insight into the genome, particularly in human beings but also that of economically important plants and animals will far outstrip what the products themselves are going to do. For example, in the general domain of human medicine, there is no disease that is not modified by the genetic composition of the host. At one extreme, we have those single gene syndromes, of which phenylketonuria or sickle cell disease are prototypic, which are almost directly determined by the genetic composition of the individual, although even there, with some insight, we can learn something about environmental amelioration that will prevent the full development of that syndrome. So there is no disease that is purely genetic even in those particular circumstances. More important public health problems are heart disease, cancer, psychiatric disease, and range of autoimmune and related syndromes. And for each of these we know that there are important genetic factors that will influence the likelihood of development of the disease, but know very little about specific genes involved, although that knowledge is increasing rapidly. I would submit that the extension of the techniques that have been introduced, for example by Y. W. Kan for prenatal diagnosis of sickle cell disease, the isolation of the DNA segment that is responsible for the determination of the structure of hemoglobin, that those techniques are already and very rapidly will be generalized to the total mapping of the human genome and the ability to recognize correlations, not between grandparents and their children or between siblings and one another, which is an extremely feeble method of genetic analysis, but correlations between the presence of a specific nucleotide sequence in a chromosome and the emergence of disease with some probability in the individual carrying it. That can then be used as a most effective hammer and wedge to work out the biochemical basis of these syndromes and the development of further therapeutic measures. Even without the benefit of the DNA technology we have already made very great strides in that direction in our elucidation of the mechanisms of deposits of cholesterol in the arteries of atherosclerosis with the studies of the metabolism of cholesterol, the identification of the carrier proteins. There is a large school of people involved in this, particularly in the work of Brown and Goldstein, looking at rare anomalies in cholesterol deposition and how to pry loose basic mechanisms of cholesterol transport by the lipid protein systems of the blood; their dissection and analysis have given us our most important lead on the actual

pathogenetic mechanisms involved in that disease. At that point we leave the genetics of rare diseases; we have used genetic insight as a method of biochemical and physiological analysis, and I think one can expect very great promise from this approach in that context and as well in the other major public health problems.

Now on a somewhat mixed area of insight and products, I would put that the most telling challenge that we face in this field is the systematic survey of the approximately 100,000 different proteins of which the human organism is constituted again using the kinds of technology which are available to us at the present time. We know of that set a few dozen that have been characterized well, that is to the extent that we have got the amino acid sequences and the crystal structures and so on, and we have a reasonable awareness of perhaps 100 to which names can be attached; we can talk about a specific entity that constitutes an enzyme or a structural protein or similar functional constituent, perhaps a couple of hundred might be put in that category if we include some of the polypeptides. So you can see we have barely scratched the surface, because in order to approach that kind of dissection we have generally had to relate a structure and a function simultaneously; if we are talking about an entity which is present to the extent of less that 1 per 1000 of the total protein composition of a cell, you have to know what you are looking for in order to be able to isolate it; you are not going to get at it by gross chromatographic separation, you simply do not have enough material available using our present techniques. The cloning of the corresponding messengers is in principle a pretty straightforward task for developing this library or cataloging of the essential constituents in relation to the particular organisms that they come from, and a variation of functional states can be examined at the level of the messengers, and I think this becomes probably our most important new tool in the examination of human physiology and biochemistry starting from that genetic perspective.

Of those 100,000 proteins, we already know only a modest sprinkling, perhaps half a dozen, well enough to know that they have a therapeutic application. These are some of the biologicals that have been produced for some time, insulin; growth hormone; interferon is emerging in this context. We use a very dirty material, because we have no better, for prophylaxis for hepatitis, so we call it mixed human gamma globulin. We use a slightly cleaner stuff, which may be 1 per 1000 pure and call it RhoGamma ^{R} and use it as a prophylaxis for Rh disease. And then we have the hemophilia proteins and there are a few more. And there I have almost exhausted the human biologicals which are available today as therapeutic entities, because we are trapped in a vicious cycle of not knowing about them because we didn't have them, not being able to validate the therapeutic efficacy until they are available at some point. And then in many cases, as for example, human growth hormones, we are facing almost insuperable difficulty in getting enough material from human sources in order to really try them out effectively for therapeutic purposes, so there is a great deal about the biology and the therapeutics that has simply not been accessible to us in that way.

We will hear more headlines in future like the interferon story; I think it has become quite evident that that is an exciting wrinkle; but there really is no firm reason to believe that it is the panacea that one would like to observe; there is nothing in the history of

the development of the subject to give one confidence that it was likely to be or to have been the major answer to the problem of the control of cancer. It is a member of a considerable class of substances of which we are only slightly more than vaguely aware, that regulate the growth and function of cells in the immune system and macrophage system and whether it, or any one of a number of several hundred alternatives, is going to end up being the final candidate for the most efficacious application remains to be seen. So the door has been opened up a crack and has given us a prototype of a very large family of potential alternatives, and we have now a way to break the vicious cycle of finding enough of these substances and producing them in sufficient quantity to try them out, to be able to make our deliberate choices as to which are the candidates for more extensive trials.

Biologicals, generally, have always posed some rather difficult problems of certification, validation, reliability, purity and safety. In many cases, we use what we have because they were the best that seemed to be available, although I question whether we couldn't have gone further in areas like vaccines, which are terribly dirty materials even today, although they are far better than they were 5 and 10 years ago. There is no reason in the world why those should not be produced as chemically and biologically homogeneous materials and with some prospect both of greater efficiency in vaccination and with some possibility avoiding some of the more serious side effects certainly, we should be able to avoid the more trivial ones of response to contaminating proteins and so on. Similar considerations apply to the gamma globulins that we use today; pooled human blood as a source of material without specific fractionation for chemical entity - I think we will look back 10 or 15 years from now and wonder how we could have been as barbarous as we have been now in using materials of that kind, but one can say we didn't have many alternatives. But those are the alternatives, of course, that are most evidently opened up now to DNA technology because those are products that we know have a therapeutic application - there is no problem about discovering a market for them, we simply have to go out and learn how to produce these:

I would just like to put in a note of hope that we have a technology that is not only economic and efficient and rational, but gives us the opportunity to examine these issues of validity and certification and identity and purity to a degree that we have not had before, And certainly part of any program for the production of a human protein using a microbial fermentative process ought to have built into it the demonstration of how you know that the stuff you make tomorrow is the same that you made yesterday, in your trials with it, in respect to its homogeneity. And then the delightful point is that producing it through such a rational approach, we really do have the fundamental tools to answer that question. It is going to be in fact easier to demonstrate the homogeneity of the genetic information in a clone of organisms being used in a fermentation than it is to demonstrate the chemical homogeneity of the protein product. I think we are going to find that there will be mutated insulins and mutated interferons that might or might not be consequential when introduced chronically into human subjects, that would pose great difficulty unless you knew what you were looking for in identifying them in parts per hundred contamination, and would not be difficult at all to demonstrate that at the level of genetic composition of the organ-

isms producing them. But it is my hope that if these issues are opened up early, that the appropriate and entirely feasible methodology will be built in from the very start and prevent the kind of aberration that could result in a backlash against the entire field.

If we go to other lines of application of these technologies (and here I will be treading on ground that is much more familiar to many of you than it is to me), I think we run into some problems about the way that our economy is structured, that may provide very great encouragement to some of the wonderful things I have just been talking about in medicine, but do not provide quite the same degree of support in other important areas. I am rather discouraged in particular by agricultural innovation, because the development of new crops does not have built into it the same kind of entrepreneurial incentive as applies to the industrial framework that we are familiar with here. I don't know exactly what can be done about that. It does make it an area for perhaps more direct government, that is socialized, intervention in order to meet social needs, but I think it is a matter that we should all be concerned about. Similarly, we already have had proposals, and I think they are just the beginning, about the way in which restructured organisms could be absolutely indispensable in environmental clean-up, and there again the problem of how to get return on investing in development of such a business in the private sector. This is a serious obstacle to eliciting the same kind of ingenuity and industry and diligence and capital investment and so forth that we do have on the productive industrial side. So I think these are some of the structural problems that we should be thinking about and how to make the most use of these technologies.

Resource conversion is in a somewhat intermediate state in that respect; it is hard to see how you are going to make a business out of efficient garbage disposal except by heavy taxes on present effluents, although of course our sewage plants already reflect a certain ingenuity in using what nature has to offer us by way of microbial intervention. There will be a few commodity chemicals that will emerge as new products not now being made, not merely improvements over existing procedures, but I guess I remain somewhat skeptical whether fermentation systems are really going to be as important in biomass conversion, in producing fundamental feedstocks and so on, as they are in the production of specialty materials of very intricate structure of the kind that we have indicated before. There will be very important exceptions to that, but the question remains if you are dealing with materials of rather simple organic structure, whether the organic chemists and the application of physical and chemical methods of conversion and the handling efficiency and the rates of conversion that one can get at higher temperatures, the use of specialized reagents and so on, isn't going to overtake what we have put in this field. That is something that is susceptible to alternative argument. One would like to see more fundamental analyses of process cost and process flow, not only in terms of what is done now, but what are the horizons, what are the margins, what are the limitations for conversion processes along these lines? I think we need those as strategic markers in order to indicate what feasible technology there is going to be in particular areas.

I also think that industrial microbiologists ought to be looking over their shoulders about competition from other organisms to conduct some of the conversions that are certainly much easier to program and much easier to initiate using microbes. Again, if I think about the process handling that is involved in the production of a specialty protein, I wonder if I wouldn't rather grow a few plants in my garden in order to produce a kilogram of some protein modification that might be represented in the seeds, than to establish a sterile fermentation tank to yield exactly that result. This is not the large-scale manipulation of crops, the development of a dwarf rice or whatever; but it is asking if we can go as far as we have gone, and then one step further, into the modification of the plant genome, wouldn't we solve a lot of production problems with microbes? My preference would be something you could graft onto a tree in order to produce specialty outputs. The same generalization might apply to a class of organisms that has been grossly underestimated, except in some primitive, indigenous cultures; and these are invertebrates like termites, earthworms and so forth which have enormous capability for biomass conversion and which have the capacity for mechanical handling of those materials that may exceed what we can do economically with a ball mill and so on.

One of the fundamental challenges that faces us now altogether is the very pace of innovation; the field is growing so explosively that the pace of new scientific and technical insight exceeds by far the rate at which new processes can be brought to market, and by still a further order of magnitude, the pace at which it is possible to get through the regulatory controls that those materials that are involved in human medicine will certainly have to face. And that may be one of the most important strategic decisions that this community has to address. Anything that you start now will take five years before you can bring it to term - what are the chances that it is going to be obsolescent by the time you are even half-way there? And I think in many areas the changes are very high. I think the only possible answer to this is a rather integrated approach to the development of the field that is not just devoted to creamskimming of specific scientific opportunities, but which is very thoughtful about the integration of the academic and scientific and technical aspects of these developments. To do that properly, in my view, is going to require development of new systems for the interaction of the academic and the industrial and the government world, which must be pursued within the framework of the strategic issues I have just begun to discuss. Thank you.

1. In January 1981, Karl Illmensee and Peter Hoppe reported the successful transplantation of nuclei from embryo cells to eggs in mice.